Available Online at

http://www.ijcpa.in

IJCPA, 2015; 2(3): 147-151

International Journal of CHEMICAL AND PHARMACEUTICAL ANALYSIS

eISSN: 2348-0726 ; pISSN : 2395-2466

Research Article

SYNTHESIS OF AMINOACETAMIDO SUBSTITUED CHALCONE FOR ANTIBUERCULAR, ANTIMICROBIAL AND ANTI INFLAMMATORY ACTIVITY

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Received: 21 December 2014 / Revised: 5 January 2015 / Accepted: 29 March 2015 / Online publication: 1 July 2015

ABSTRACT

The various pharmacological properties of chalcones has instigated us to synthesis and study antitubercular antimicrobial and anti inflammatory activity. Following Claisen-Schmidt condensation reaction, chloroacylaminochalcones (**4a-b)** were obtained by the reaction of chloroacetylamido acetophenone with substituted benzaldehyde, treatment of (**4a-b)** with substituted amines yielded the desired compound**.** Structures of synthesized compounds were established by spectral data's. Antitubercular activity was determined by Microplate Alamar Blue Assay Method against *M. tuberculosis* H37 RV and *in vitro* anti inflammatory study was conducted by bovine serum albumin assay method. Except **5c** compound all have shown anti tubercular effect at 100 µg /ml and 50 µg /ml,and were sensitive to microbial strains used and inhibition denaturation of protein was observed at 10 µg /ml.

Keywords: Chalcone, Anti tubercular, Anti-inflammatory, Antibacterial activity.

1. INTRODUCTION

Tuberculosis (TB) is a bacterial infection and is becoming alarming due to the presence of multidrug resistance. The WHO has estimated that nearly a billion to be infected by 2020. The regeneration of TB is closely linked to the emergence of HIV and total deficiency of the immune system. Despite enormous efforts, no new drug has been introduced in the market for the past 40 years[1]. Effective treatment of microbial infections is challenging for various reasons including: lack of accessibility and elevated expenses of drugs and low adherence owing to toxicity of second-line drugs. For this purpose, new antimicrobial agents are urgently needed, and research programs for alternative therapeutics should be encouraged. The inevitable consequence of the widespread use of antimicrobial agents has been the emergence of antibiotic resistance pathogens, fueling an ever-increasing need for new drugs.

Non-steroidal anti-inflammatory drugs are commonly prescribed for the treatment of acute and chronic inflammation, pain and fever. However, long term clinical usage NSAID's is associated with significant side effects of gastric lesions, bleeding and nephrotoxicity. Therefore, the discovery of new safer anti-inflammatory drugs represents a challenging goal for such a research area.

Chalcones are secondary metabolite precursors of flavonoids and isoflavonoids, which are commonly found in edible plants. The presence of flavonoids in fruits and vegetables has been related to the health benefits. A good safety profile and easy of synthesis are the factors contributing to the increasing interest in exploring the pharmacological activities of chalcones.

Chalcones can be viewed as bifunctional molecule imbibed with a keto group and a conjugated double bond. Synthesis of this versatile molecule can be carried out easily and conveniently by Claisen-Schmidt condensation reaction in which acetophenone

and benzaldehyde and their derivative are reacted in the presence of aqueous alkali.Chalcones have been reported to exhibit several biological activities including antitumor[2,3] anti-inflammatory,[4], antibacterial,[5,6], antimalarial[7], antitubercular[8], antioxidant [9] and antispasmodic activities[10].in the present study an attempt is made to synthesis novel chalcone derivatives and screen for different biological activity.

2 MATERIALS AND METHODS

2.1 Materials

The chemicals and reagents used were of AR and LR grade, procured from Finar, S.D- Fine Chem. Ltd. UV spectra of the synthesized compounds were recorded on UV-Visible spectrophotometer (Shimadzu 1601) using methanol and the values of wave length (λ max) were reported in nm. The IR spectra of the synthesized compounds were recorded on a Fourier Transform IR Spectrophotometer (model Shimadzu 8700) .1H-NMRand 13C NMR spectra were recorded on Bruker Avance II 400 NMR spectrometer using DMSO and chemical shift (δ) was reported in parts per million downfield from internal reference Tetramethylsilane.

2.2 Experimental

2.2.1 Chemistry

Synthesis of *N*-(4-Acetyl-phenyl)-2-chloro-acetamide (3)

4-amino acetophenone and glacial acetic acid were taken stirred well in warm water bath. A solution of chloroacetyl chloride in glacial acetic acid was prepared which was added to above reaction mixture drop wise with constant stirring. After addition was complete, stirring was continued for 30 minutes then add 0.4 M Sodium acetate solution, the precipitate obtained was cooled in ice water bath for 5 minutes, washed with water. Crude product was re-crystallized from ethanol.

Synthesis of 2-Chloro-*N*-{4-[3-(4-methoxy-phenyl)-acryloyl]-phenyl} - acetamide. (4 a-b)

Equimolar quantity of *N*-(4-Acetylphenyl)-2-chloro-acetamide and substituted benzaldehyde were dissolved in ethanol and 20% NaOH solution was added slowly with stirring. After complete addition stirring was continued for 6 hours and kept overnight. The reaction mixture was decomposed in ice-water, and acidified with 10 % HCl to obtain product. Crude product was re-crystallized from ethanol.

Synthesis of substituted 2-amino-N-{4-[3-(4-methoxy-phenyl)-acryloyl]-phenyl} acetamide 5(a-f)

Equimolar quantity of 2-Chloro-*N*-{4-[3-(4-methoxyphenyl)-acryloyl]-phenyl} - acetamide and respective amines were dissolved in 30 ml of ethanol and refluxed for 3 hours. Reaction mixture is cooled at room temperature and poured into crushed ice, crude product obtained was re-crystallized from ethanol.

***N*-{4-[3-(4-Methoxy-phenyl)-acryloyl]-phenyl}-2-(4-nitro-phenylamino) - acetamide. (5 a)**

Yellowish brown , M.P – 136-38^{0}C , % yield 46.97, λmax 209 nm , IR(KBr)(cm^{-1}) 3470 (NH), 2924 (CH_2), 1633 (C=O), 1589 (CONH), 1469 (C=C), 1027 (C-O) ; .^{1}HNMR (DMSO)- δ 8.04, m, 2H, NH; δ 7.91, d, 1H, CH J=8.6Hz; δ 7.57, d, 1H, CH, J=8.76 Hz ; δ 6.7-8.08, m,13H, Ar H; δ 4.3, s, 1H, NH; δ 3.9, s, 3H OCH_3; δ 1.25, s, 2H, CH_2.13C NMR(DMSO) δ 180, C=O, δ160,C=O, δ143,C=C, δ126 C=C, δ 61,C.

2-(4-Chloro-phenylamino)-N-{4-[3-(4-methoxy-phenyl)-acryloyl]-phenyl} - acetamide. (5 b)

Yellow. M.P – 140-42 ^{0}C. . % yield 65.00; λ max – 203.00 nm IR (KBr) (cm^{-1}) 3446 (NH), 2918 (CH2), 1593 (C=O), 1508 (C=C Ar), 1429 (C=C), 1294 (C-O), 615 (C-Cl). ^{1}HNMR (DMSO- δ ppm) δ 8.04, m, 1H, NH; δ 7.91, d, 1H, CH J=8.6Hz; δ 7.57, d, 1H, CH,J=8.76Hz ; δ 6.7-8.08, m,13H, Ar H; δ 4.3, s, 1H,NH; 3.9, s, 3H,OCH_3; δ 1.25, s, 2H,CH_2.

2-Hydrazino-N-{4-[3-(4-methoxy-phenyl)-acryloyl]-phenyl}-acetamide (5 c)

Yellowish brown, M.P. – 116-180°C, % yield 70.33, λmax-209 nm , IR(KBr)(cm^{-1}) 3431,3371(NH str), 1595 (C=O), 1448(C=C), 1300 (C-O) ; ^{1}HNMR (DMSO- δ ppm) 8.2,s,1H,NH; δ 7.57, d, 1H, CH,J=8.76Hz ; δ 7.91, d, 1H, CH J=8.6Hz; δ 6.79-8.08,m,13H, Ar H ; δ 4.3, s, 1H NH; 3.9, s, 3H, OCH_3 ; δ 1.25, s, 2H,CH_2.

2-(4-Nitro-phenylamino)-N-{4-[3-(3, 4, 5-trimethoxy-phenyl)-acryloyl-phenyl}-acetamide (5d)

Yellow, M.P 118-200 ˚C, % yield 38.52, λmax 207.80nm. IR (KBr) (cm-1**)** 3470 (NH), 2926(CH_2), 1622 (C=O), 1589 (CONH), 1456 (C=C), 1170 (C-O). ^{1}HNMR (CDCl3) δ 8.04, m, 1H, NH ; δ 7.91, d, 1H, CH J=8.76Hz; δ 7.57, d, 1H, CH J=8.6Hz; δ 7.92-7.95, m, 2H, ArH ; 7.62- 6.69,m, 8H,ArH; δ 4.5, s, 2H, CH_2; δ 4.2, s, 1H, NH ; δ

3.9, m, 9H, OCH_3 ; 13C NMR(DMSO) δ 180, C=O, δ160,C=O, δ143,C=C, δ126 C=C, δ 61,C.

2-(4-Chloro-phenylamino)-N-{4-[3-(3,4,5-trimethoxy-phenyl)-acryloyl]-phenyl}-acetamide (5e) Yellow M.P 160-62 ^{0}C, % yield 52.17, λmax - 210.00 nm IR(KBr)(cm^{-1}) 3464 (NH), 3221 (C-H Ar), 3340 (NH), 2933 (CH_2), 1583 (C=O), 1454 (C=C), 1280 (C-O), 586(C-Cl)

Hydrazino-N-{4-[3-(3, 4, 5-trimethoxy-phenyl)-acryloyl]-phenyl} - acetamide (5 f)

Brown, M.P – 120-220°C, % yield 67.70, λ max – 207.80nm, IR (KBr)(cm^{-1}) 3431,3371(NH), 1595 (C=O), 1448(C=C), 1300 (C-O).

2.2.2 Antitubercular **activity**

The study was conducted by adopting Microplate Alamar Blue Assay Method[11] using the strain *M. tuberculosis* H37 RV. The 96 wells plate received 100 µl of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate. The final drug concentrations tested were 100 to 0.2 µg/ml. Plates were covered and sealed with parafilm and incubated at 37 ºC for five days. After this time, 25µl of freshly prepared 1:1 mixture of Almar Blue reagent and 10 % tween 80 was added to the plate and incubated for 24 hrs. A blue color in the well was interpreted as no bacterial growth, and pink colour was scored as growth. The MIC was defined as lowest drug concentration which prevented the colour change from blue to pink.

2.2.3 Anti-inflammatory activity

Bovine Serum Albumin Method (BSA) was followed to test anti inflammatory activity[12]. A solution of 0.2 % w/v BSA was prepared in Tris buffer saline and pH was adjusted to 6.8 using glacial acetic acid. Stock solutions of 1000 µg/ml for all compounds were prepared by using methanol as solvent. Two different concentrations of 100 and 200 µg /ml were prepared by using methanol as solvent, to the concentrations prepared 0.1 ml and 5 ml of 0.2 % w/v BSA was added. The control consists of 5 ml of 0.2 % w/v BSA solution with 0.1 ml methanol. For the positive standard Indomethacin (100 µg/ml) a solution of 0.1 ml was taken in methanol and added 5 ml 0.2% w/v BSA solution . The reaction mixture was heated at 72°C for 5 minutes and then cooled for 10 min. The absorbance of these solutions was determined by using spectrophotometer at a wavelength of 660 nm. The percentage inhibition of precipitation (denaturation of protein) was determined as a using the following formula:

% Inhibition of denaturation =

$$\frac{\text{(Abs. of control – Abs of sample)} \times 100}{\text{Abs. of control}}$$

2.2.4 Anti-bacterial activity:

This study was performed by Agar diffusion (cup plate) method using the following Bacterial strain[13.]

Test organism: Bacteria NCIM Type

1. *Staphylococcus aureus* 2079 (Gram +ve)
2. *Bacillus subtilis* 2920 (Gram +ve)
3. *Escherichia coli* 2931 (Gram –ve)
4. *Klebsiella pneumonia* 2957 (Gram –ve)

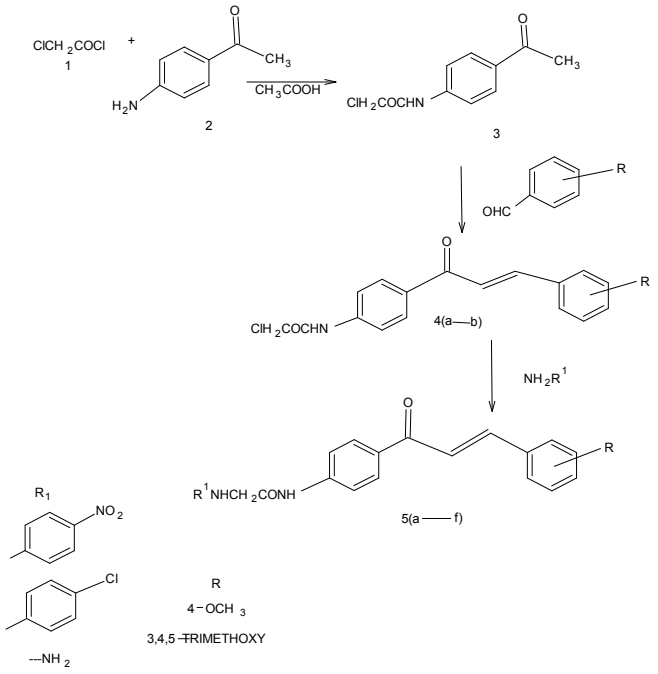
3. RESULTS AND DISCUSSION

All the chalcones derivatives were tested for antitubercular activity at concentration of 100 µg/ml to 0.8 µg/ml against the *M. tuberculosis* H37 RV. All the derivatives exhibited activity at 100 µg/ml and 50 µg/ml except compound **5(C).**

The *in-vitro* anti-inflammatory activity was calculated as % inhibition of denaturation of protein compounds were tested at 10 and 50 µg/ml and Indomethacin was used as standard and BSA solution was used as control (0.2%).The results are depicted in following table 1, it was found all compounds were effective at low concentration.

All the synthesized compounds were tested for antibacterial activity by Agar diffusion method at 100 µg/ml concentration, using Ampicillin at 10 µg/ml was used as positive control. The activity was measured as Zone of inhibition and expressed in mm (see table 2). Moderate activity was observed. The results of biological activity suggest that chalcone moiety with electron withdrawing group substituted on aryl ring would be responsible for activitiy. Reaction was carried out between chloroacetyl chloride with 4-amino acetophenone to obtain chloroacetylamido acetophenone which was subjected to Claisen Schmidt condensation with substituted benzaldehyde to obtain chloroacylaminochalcones derivatives;these derivatives were treated with different amines to obtain substituted aminoacetamidochalcones 5(a-f). The formation of chloroacylamino chalcones derivatives was proved from the

spectral data. IR absorption showed carbonyl peak at 1639 cm-1, olfenic bond of propenone at 3064 cm-1 and acylamino NH at 3437 cm-1 respectively. The formation oftitled compounds were proved by their spectral data's, The presence of propenone moiety is supported by 1H NMR ($CDCl_3$), doublet peaks were seen at δ 7.6 and 7.4 for olefinic protons the same supported by ^{13}C NMR shift seen at δ 126 and 143 respectively. The presence of propenone oxo group can be proved by the absorption peak at 1622 cm-1 and same at δ 180 from carbon spectra. The formation of substituted aminoacetamido of derivatives was be proved by singlet methylene peak seen at δ 4.5 and δ 61.01, the carbonyl carbon at δ 160; the broad peak at δ 4.26 for NH and the same supported by IR absorption seen at 3470 cm^{-1} from the respective spectra's, the aryl carbon and protons were seen in the expected region.



Scheme-1: Schematic presentation of synthesis of chalcone derivatives

Table 1: Anti-inflammatory activity of novel chalcones

Compounds	Percentage Inhibition	
	10 µg/ml	50 µg/ml
5(a)	7.25	-
5(b)	20.47	-
5(c)	15.14	-
5(d)	-	-
5(e)	45.58	-
5(f)	34.82	-
Indomethacin	92	96

Table 2: Antibacterial activity of novel chalcones

Compound Code	Zone of Inhibition (mm)			
	S. aureus	***Bacillus subtilis***	***E. Coli***	***K. Pneumonia***
5 (a)	-	12	8	9
5b)	11	9	7	9
5(c)	6	8	8	7
5(d)	7	8	-	9
5(e)	-	7	6	7
5(f)	7	10	7	7
Ampicillin	14	20	15	14

4. CONCULSION

The present study describes the synthesis of 2-amino-N-{4-[3-(4-methoxy-phenyl)-acryloyl]-phenyl} acetamide. All the compounds have been obtained in good yields and purity. The structure of the compounds was confirmed by IR, NMR, and mass spectral data. All the derivatives were evaluated for antitubercular, antimicrobial and anti-inflammatory activities and it was found that the compounds have exhibited moderate to good activity.

ACKNOWLEDGMENT

The authors wish to thank Mr. Manish, Panjab University, Chandigarh for providing the spectral data and Dr Kishore G Bhat, Professor Department of Biotechnology, MM'S Halgekar Institute of Dental Sciences and Research Centre, Belgaum for screening the synthesized compounds for antitubercular activity.

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